

**Exhibit A**



U.S. Patent Application Serial No. 08/974,584  
For: TELOMERASE REVERSE TRANSCRIPTASE  
Filed: November 19, 1997  
Inventors: Cech, et al.

**PENDING CLAIMS AFTER ENTRY PRELIMINARY AMENDMENT**

1. An isolated, substantially pure, or recombinant protein preparation of a telomerase reverse transcriptase protein, or a variant thereof, or a fragment thereof.
2. (Amended) An isolated, substantially pure, or recombinant telomerase reverse transcriptase protein, said protein characterized by having an amino acid sequence with at least 75% sequence identity to the telomerase reverse transcriptase protein that has a sequence set forth in SEQ ID NOS:2, 4-6, 52, 58, 63, 64, 65, 67, or 69, or a variant thereof, or a fragment thereof.
3. (Amended) An isolated telomerase reverse transcriptase protein, or a variant thereof, or a fragment thereof, said protein:
  - (i) having a calculated molecular weight of about 50 to 150 kDa; and
  - (ii) (a) specifically binding to an antibody raised against a protein, or immunogenic fragment thereof, that has a sequence set forth in SEQ ID NO:NO:2, 4-6, 52, 58, 63, 64, 65, 67, or 69; or
  - (b) having 60% amino acid sequence identity to a protein that has a sequence set forth in SEQ ID NO:NO:2, 4-6, 52, 58, 63, 64, 65, 67, or 69.
4. (Amended) The protein of claims 1, 2 or 3 that has the sequence set forth in SEQ ID NOS:2, 4-6, 52, 58, 63, 64, 65, 67 or 69, or a variant thereof or a fragment thereof.
5. The telomerase reverse transcriptase protein of claims 1, 2 or 3 wherein the protein is produced by recombinant means or synthetic means.
6. (Amended) The telomerase reverse transcriptase protein of claims 1, 2 or 3 wherein the protein is encoded by a nucleic acid molecule which specifically hybridizes to SEQ ID NOS:1, 3, 19, 53, 62, 66 or 68.
7. The telomerase reverse transcriptase protein of claims 1, 2 or 3 wherein the protein, variant, or fragment has telomerase catalytic activity.
8. The telomerase reverse transcriptase protein of claims 1, 2 or 3 wherein the protein, variant, or fragment does not have telomerase catalytic activity.
9. The telomerase reverse transcriptase protein fragment of claims 1, 2 or 3 that comprises at least about 6 amino acid residues.

10. An isolated, synthetic, substantially pure, or recombinant polynucleotide comprising a nucleic acid sequence that encodes a telomerase reverse transcriptase protein or variant thereof or a fragment thereof.

11. (Amended) An isolated, synthetic, substantially pure, or recombinant polynucleotide selected from:

(a) a polypeptide comprising a sequence as set forth in SEQ ID NOS:2, 4-6, 52, 58, 63, 64, 65, 67 or 69;

(b) a polynucleotide of at least 10 nucleotides which hybridizes to the foregoing DNA and which codes for a telomerase reverse transcriptase protein or variant;

(c) the DNA having a sequence that encodes a polypeptide encoded by a polynucleotide of (a) or (b).

12. An isolated nucleic acid encoding a telomerase reverse transcriptase protein, said protein defined as follows:

(i) having a calculated molecular weight of between 50 and 150 kDa; and

(ii) (a) specifically binding to an antibody raised against a telomerase reverse transcriptase protein, or a immunogenic fragment thereof; or

(b) having at least 60% amino acid sequence identity to a telomerase reverse transcriptase protein.

13. An isolated nucleic acid of claims 10, 11 or 12, wherein the telomerase reverse transcriptase protein is from *Euplotes*, *Schizosaccharomyces*, *Tetrahymena*, *Oxytrichia*, mouse, or mammals.

14. The isolated nucleic acid of claims 10, 11 or 12, wherein the calculated molecular weight of the encoded telomerase reverse transcriptase protein is about 123 to 127 kDa.

15. (Amended) The isolated nucleic acid of claims 10, 11 or 12, wherein the encoded telomerase reverse transcriptase protein has at least 80% amino acid sequence identity to a protein comprising SEQ ID NOS:2, 4-6, 52, 58, 63, 64, 65, 67 or 69.

16. (Amended) An isolated nucleic acid which specifically hybridizes to SEQ ID NOS:1, 3, 53, 62, 66, or 68 under stringent conditions.

17. (Amended) An isolated nucleic acid encoding a protein that specifically binds to an antibody directed against the protein, or immunogenic fragment thereof, wherein the protein has a sequence set forth in SEQ ID NO:2, 4-6, 52, 58, 63, 64, 65, 67 or 69.

18. (Amended) An isolated nucleic acid that has a nucleotide sequence encoding at least about five contiguous amino acids of an amino acid having a sequence as set forth in SEQ ID NO:2, 4-6, 52, 58, 63, 64, 65, 67 or 69, or variants thereof.

19. An expression vector that has the nucleic acid sequence claims 10, 11, 12, 16, 17, or 18.
20. A cell comprising a polynucleotide as defined in any of the claims 10, 11, 12, 16, 17, or 18.
21. A transfected cell that has a heterologous gene coding for at least 10 nucleotides of a telomerase reverse transcriptase-encoding nucleic acid.
22. A transfected cell into which has been introduced an exogenous nucleic acid sequence which specifically hybridizes under stringent conditions to the nucleic acid of claims 10, 11, 12, 16, 17, or 18, has been introduced, and which expresses the exogenous nucleic acid as a protein or peptide.
23. The transfected cell of claim 21 or 22, wherein the transfected cell is a mortal, karyotypically normal, diploid cell.
24. The transfected cell of claim 21 or 22, wherein said cell is derived from a bacterium, an insect, a plant, a fungus, a yeast or a mammal.
25. The transfected cell of claim 21 or 22 that is contained within a non-human animal or progeny thereof.
26. A non-human animal, or progeny thereof, into which an exogenous nucleic acid sequence which specifically hybridizes under stringent conditions to the nucleic acid of claims 10, 11, 12, 16, 17, or 18, has been introduced and the animal expresses the exogenous nucleic acid as a telomerase reverse transcriptase protein or fragment thereof.
27. A transgenic non-human animal that has a heterologous gene coding for at least 10 nucleotides of a telomerase reverse transcriptase protein-encoding nucleic acid.
28. The non-human animal of claim 26 or 27, wherein the animal is a mouse.
29. The non-human animal of claim 26 or 27, wherein said animal comprises a recombinant telomerase reverse transcriptase gene that differs from a naturally occurring telomerase reverse transcriptase gene in one or more codons.
30. The non-human animal of claim 29, wherein said gene differs from a naturally occurring telomerase reverse transcriptase gene by having a substitution, missense mutation, a nonsense mutation, an insertion, or a deletion.

31. The non-human animal of claim 26 or 27, wherein said animal is deficient in a telomerase activity.
32. The non-human animal of claim 31, wherein said deficiency is a result of a gene encoding a telomerase having a reduced level of a telomerase activity compared to a wild-type telomerase.
33. An antibody, or binding fragment thereof, wherein the antibody or fragment specifically binds to a telomerase reverse transcriptase protein or immunogenic fragment thereof.
34. (Amended) An antibody, specifically immunoreactive under immunologically reactive conditions, to a telomerase reverse transcriptase protein, or immunogenic fragment thereof, said protein having an amino acid sequence as set forth in SEQ ID NO:NO:2, 4-6, 52, 58, 63, 64, 65, 67, or 69.
35. An antibody, specifically immunoreactive under immunologically reactive conditions, to a telomerase reverse transcriptase protein, or immunogenic fragment thereof, comprising the protein encoded by the nucleic acid of claims 10, 11, 12, 16, 17, or 18.
36. The use of a polynucleotide that is at least ten nucleotides to 10 kb in length and comprises a contiguous sequence of at least ten nucleotides that is identical or exactly complementary to a contiguous sequence in a naturally occurring telomerase reverse transcriptase gene or telomerase reverse transcriptase mRNA in assaying or screening for a telomerase reverse transcriptase gene sequence or telomerase reverse transcriptase mRNA.
37. The use of a polynucleotide that is at least ten nucleotides to 10 kb in length and comprises a contiguous sequence of at least ten nucleotides that is identical or exactly complementary to a contiguous sequence in a naturally occurring telomerase reverse transcriptase gene or telomerase reverse transcriptase mRNA in preparing a recombinant host cell.
38. A method of determining whether a compound or treatment is a modulator of a telomerase reverse transcriptase activity or expression comprising detecting a change in activity or expression in a cell, animal or composition comprising a telomerase reverse transcriptase recombinant protein or polynucleotide following administration of the compound or treatment.
39. A method of determining whether a test compound is a modulator of a telomerase reverse transcriptase activity, said method comprising the steps of;
  - (a) contacting a telomerase reverse transcriptase of claims 1, 2, or 3, with the test compound; and
  - (b) measuring the activity of the telomerase reverse transcriptase, wherein a change in the telomerase reverse transcriptase activity measured in the presence of the test

compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the telomerase reverse transcriptase activity.

40. A method of preparing recombinant telomerase, said method comprising contacting a recombinant telomerase reverse transcriptase of claims 1, 2, or 3, with a telomerase RNA component under conditions such that said recombinant protein and said telomerase RNA component associate to form a telomerase enzyme capable of catalyzing the addition of nucleotides to a telomerase substrate.

41. A method of detecting a telomerase reverse transcriptase gene product in a sample comprising:

- (a) contacting the sample with a probe that specifically binds the gene product, wherein the probe and the gene product form a complex, and detecting the complex; or
  - (b) specifically amplifying the gene product in the biological sample, wherein said gene product is a nucleic acid, and detecting the amplification product;
- wherein the presence of the complex or amplification product is correlated with the presence of the telomerase reverse transcriptase gene product in the biological sample.

42. A method of detecting the presence of at least one telomerase positive human cell in a biological sample comprising human cells, said method comprising the steps:

- (a) measuring the amount of a telomerase reverse transcriptase gene product in said sample,
  - (b) comparing the amount measured with a control correlating to a sample lacking telomerase positive cells,
- wherein the presence of a higher level of the telomerase reverse transcriptase gene product in said sample as compared to said control is correlated with the presence of telomerase positive cells in the biological sample.

43. A method for diagnosing a telomerase-related condition in a mammal, comprising:

- (a) obtaining a cell or tissue sample from the mammal;
  - (b) determining the amount of a telomerase reverse transcriptase gene product in the cell or tissue; and
  - (c) comparing the amount of telomerase reverse transcriptase gene product in the cell or tissue with the amount in a healthy cell or tissue of the same type;
- wherein a different amount of telomerase reverse transcriptase gene product in the sample from the mammal and the healthy cell or tissue is diagnostic of a telomerase-related condition.

44. A method for increasing the proliferative capacity of a vertebrate cell in vitro by increasing expression of telomerase reverse transcriptase in the cell.

45. The use of an agent which increases the expression or activity of a telomerase reverse transcriptase in the manufacture of a medicament for the treatment of a condition addressed by increasing proliferative capacity of a vertebrate cell.
46. The use defined in claim 45 wherein the medicament is for inhibiting an effect of ageing.
47. A pharmaceutical composition comprising an acceptable carrier and a telomerase reverse transcriptase protein, variant or fragment of claims 1, 2, or 3, a reverse transcriptase antibody or binding fragment of claims 33, 34, or 35, a polynucleotide encoding a telomerase reverse transcriptase protein, variant or fragment as defined in claims 10, 11, 12, 16, 17, or 18, or a nucleic acid that encodes a telomerase reverse transcriptase protein or subsequence thereof.
48. The use of an inhibitor of telomerase expression or activity in the manufacture of a medicament for the treatment of a condition associated with an elevated level of telomerase activity within a mammalian cell, said inhibitor being a polynucleotide of claims 10, 11, 12, 16, 17, or 18, or a polypeptide of claims 1, 2, or 3, or a compound discovered using any of the foregoing.
49. A protein, variant or fragment of any one of claims 1, 2, or 3, for use as a pharmaceutical.
50. The use of a protein, variant or fragment of any one of claims 1, 2, or 3, in the manufacture of a medicament.
51. The use of a protein, variant or fragment of any one of claims 1, 2, or 3, in the manufacture of a medicament for inhibiting an effect of ageing or cancer.
52. A polynucleotide or fragment of claims 10, 11, 12, 16, 17, or 18 for use as a pharmaceutical.
53. The use of a polynucleotide or fragment of claims 10, 11, 12, 16, 17, or 18 in the manufacture of a medicament.
54. The use of a polynucleotide or fragment of claims 10, 11, 12, 16, 17, or 18 in the manufacture of a medicament for inhibiting an effect of ageing or cancer.
55. The use of claims 54 wherein the medicament's inhibitory effect on ageing increases the lifespan of a cell or an animal to which the medicament is administered.
56. A method for detecting the presence of a polynucleotide sequence encoding at least a portion of a telomerase reverse transcriptase in a biological sample, comprising the steps of:

- (a) providing:
    - (i) a biological sample suspected of containing a nucleic acid corresponding to the polynucleotide sequence of a telomerase reverse transcriptase;
    - (ii) a probe comprising a nucleotide sequence of a telomerase reverse transcriptase, or a fragment thereof capable of hybridizing to a telomerase reverse transcriptase from a biological sample;
  - (b) combining said nucleic acid-containing biological sample with said probe under conditions such that a hybridization complex is formed between said nucleic acid and said probe; and
  - (c) detecting said hybridization complex.
57. The method of claim 56, wherein, said nucleic acid in said biological sample is ribonucleic acid.
58. The method of claim 56, wherein said detected hybridization complex correlates with expression of a telomerase reverse transcriptase in said biological sample.
59. The method of claim 56, wherein, said nucleic acid in said biological sample is deoxyribonucleic acid.
60. The method of Claim 56, wherein said detecting of said hybridization complex comprises the detection of alterations in the nucleotide sequence of a telomerase reverse transcriptase in said biological sample.
61. The use of a polynucleotide or fragment comprising a purified antisense nucleotide having a nucleic acid sequence complementary to at least a portion of the telomerase reverse transcriptase polynucleotide of claim 10, 11, 12, 16, 17, or 18, in the manufacture of a medicament.
62. (Amended) The use of claim 61, wherein the polynucleotide or fragment has a nucleic acid sequence complementary to the nucleic acid sequence set forth in SEQ ID NO: 1, 3, 53, 62, 66 or 68.
63. The use of claim 61 wherein the polynucleotide is inserted in a recombinant expression vector.

64. A method for producing a polypeptide comprising an amino acid sequence encoded by a nucleotide sequence of claim 10, 11, 12, 16, 17, or 18, the method comprising culturing a host cell comprising a nucleotide sequence of claims 10, 11, 12, 16, 17, or 18, under conditions suitable for the expression of the polypeptide encoded therein.
65. The method of claim 64, further comprising a step wherein the polypeptide is isolated or purified.
66. A method for detecting the expression or presence of a telomerase reverse transcriptase in a biological sample comprising the steps of:
- a) providing:
    - i) a biological sample suspected of expressing telomerase reverse transcriptase protein; and
    - ii) the antibody of Claims 33, 34, or 35;
  - b) combining said biological sample and said antibody under conditions such that an antibody:telomerase reverse transcriptase protein complex is formed; and
  - c) detecting said complex wherein the presence of said complex correlates with the expression or presence of said telomerase reverse transcriptase in said biological sample.
67. An isolated polypeptide comprising at least a portion of the amino acid sequence selected from the group consisting of SEQ ID NO:4-6, or variants thereof, or fragments thereof.
68. The polypeptide of Claim 67, wherein said portion of said polypeptide comprises fragments of SEQ ID NO:4-6, having a length greater than 6 amino acids.
69. The polypeptide sequence of Claim 67, wherein said variant is a homologue derived from human cells.
70. An isolated polynucleotide sequence encoding the polypeptide of claim 67, or variants thereof, or fragments thereof.
71. The polynucleotide sequence of Claim 70, comprising at least a portion of the nucleic acid sequence of SEQ ID NO:3, or variants thereof, or fragments thereof.
72. The polynucleotide sequence of Claim 70, wherein said portion of said polynucleotide comprises fragments of SEQ ID NO:3 having a length greater than 10 nucleotides.
73. The polynucleotide sequence of Claim 70, wherein said variants is a homologue derived from human cells.



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74. A telomerase enzyme complex comprised of i) a purified telomerase reverse transcriptase protein subunit, or variants thereof; ii) a purified 43 kDa telomerase protein subunit or homologue thereof, or variants thereof, and iii) a purified telomerase RNA.

75. The telomerase complex of Claim 74, wherein said telomerase reverse transcriptase protein subunit is obtained from a member of the group consisting of Euplotes, Schizosaccharomyces, yeast, Tetrahymena, fungi, mouse and mammals.

76. (Amended) The telomerase complex of Claim 74, wherein said telomerase reverse transcriptase protein subunit is encoded by a nucleic acid having a sequence as set forth in SEQ ID NO:1, 3, 53, 62, 66 or 68.

77. The telomerase complex of Claim 74, wherein said telomerase complex is capable of full or partial telomerase activity.

78. The telomerase complex of Claim 74, wherein said telomerase complex is capable of replicating telomeric DNA.

79. The telomerase complex of claim 74, wherein said 43 kDa telomerase protein subunit homologue or variant is derived from human cells.

80. A composition comprising a telomerase reverse transcriptase protein of claims 1, 2, or 3, and an RNA.

81. The composition of claim 80, wherein said RNA is a telomerase RNA (TR).

82. The composition of claim 80, wherein the Telomerase reverse transcriptase protein and the TR form a ribonucleoprotein complex with a full or partial telomerase activity.

83. Substantially pure telomerase comprising telomerase reverse transcriptase and TR.

84. The telomerase of claim 83 that is at least about 95% pure.

85. The telomerase of claim 83 that has been isolated from a cell.

86. (Amended) An isolated telomerase reverse transcriptase polypeptide comprising an amino acid sequence comprising a motif of contiguous amino acids selected from the group consisting of: (a) AA<sub>1</sub>-AA<sub>2</sub>-AA<sub>3</sub>-AA<sub>4</sub>-AA<sub>5</sub>-AA<sub>6</sub>-AA<sub>7</sub>-AA<sub>8</sub>-AA<sub>9</sub>-AA<sub>10</sub>-AA<sub>11</sub>-AA<sub>12</sub>-AA<sub>13</sub> (SEQ ID NO:464), (b) AA<sub>14</sub>-AA<sub>15</sub>-AA<sub>16</sub>-AA<sub>17</sub>-AA<sub>18</sub>-AA<sub>19</sub>-AA<sub>20</sub>-AA<sub>21</sub>-AA<sub>22</sub>-AA<sub>23</sub>-AA<sub>24</sub>-AA<sub>25</sub>-AA<sub>26</sub> (SEQ ID NO:465), (c) AA<sub>27</sub>-AA<sub>28</sub>-AA<sub>29</sub>-AA<sub>30</sub>-AA<sub>31</sub>-AA<sub>32</sub>-AA<sub>33</sub>-AA<sub>34</sub>-AA<sub>35</sub>-AA<sub>36</sub> (SEQ ID NO:466), (d) AA<sub>37</sub>-AA<sub>38</sub>-AA<sub>39</sub>-AA<sub>40</sub>-AA<sub>41</sub>-AA<sub>42</sub>-AA<sub>43</sub>-AA<sub>44</sub> (SEQ ID NO:467), and

(e) AA<sub>45</sub>-AA<sub>46</sub>-AA<sub>47</sub>-AA<sub>48</sub>-AA<sub>49</sub>-AA<sub>50</sub>-AA<sub>51</sub>-AA<sub>52</sub> (SEQ ID NO:468);

wherein, AA<sub>1</sub> is a hydrophobic amino acid or cysteine; AA<sub>2</sub> is any amino acid; AA<sub>3</sub> is any amino acid; AA<sub>4</sub> is a hydrophobic amino acid or aspartic acid; AA<sub>5</sub> is aspartic acid; AA<sub>6</sub> is a hydrophobic amino acid or tyrosine; AA<sub>7</sub> is any amino acid; AA<sub>8</sub> is any amino acid; AA<sub>9</sub> is any amino acid; AA<sub>10</sub> is a hydrophobic amino acid or tyrosine; AA<sub>11</sub> is any amino acid; AA<sub>12</sub> is any amino acid; AA<sub>13</sub> is a hydrophobic amino acid; AA<sub>14</sub> is a hydrophobic amino acid, glutamine, arginine, or glycine; AA<sub>15</sub> is any amino acid; AA<sub>16</sub> is any amino acid; AA<sub>17</sub> is any amino acid; AA<sub>18</sub> is any amino acid; AA<sub>19</sub> is any amino acid; AA<sub>20</sub> is glutamine; AA<sub>21</sub> is glycine; AA<sub>22</sub> is any amino acid; AA<sub>23</sub> is any amino acid; AA<sub>24</sub> is any amino acid; AA<sub>25</sub> is serine; AA<sub>26</sub> is any amino acid; AA<sub>27</sub> is a hydrophobic amino acid, histidine or tyrosine; AA<sub>28</sub> is any amino acid; AA<sub>29</sub> is any amino acid; AA<sub>30</sub> is any amino acid; AA<sub>31</sub> is a hydrophobic amino acid or threonine; AA<sub>32</sub> is aspartic acid; AA<sub>33</sub> is aspartic acid; AA<sub>34</sub> is a hydrophobic amino acid or tyrosine; AA<sub>35</sub> is a hydrophobic amino acid, tyrosine or lysine; AA<sub>36</sub> is a hydrophobic amino acid; AA<sub>37</sub> is glycine; AA<sub>38</sub> is a hydrophobic amino acid or cysteine; AA<sub>39</sub> is any amino acid; AA<sub>40</sub> is a hydrophobic amino acid, lysine, or threonine; AA<sub>41</sub> is any amino acid; AA<sub>42</sub> is any amino acid; AA<sub>43</sub> is any amino acid; AA<sub>44</sub> is lysine; AA<sub>45</sub> is a hydrophobic amino acid, cysteine, lysine, or tyrosine; AA<sub>46</sub> is any amino acid; AA<sub>47</sub> is a hydrophobic amino acid, tryptophan, serine, or tyrosine; AA<sub>48</sub> is any amino acid; AA<sub>49</sub> is glycine; AA<sub>50</sub> is a hydrophobic amino acid or tyrosine; AA<sub>51</sub> is any amino acid; and, AA<sub>52</sub> is a hydrophobic amino acid or arginine or glutamine.

87. (Amended) The telomerase reverse transcriptase polypeptide of claim 86, comprising an amino acid sequence comprising a motif of contiguous amino acids consisting of AA<sub>1</sub>-AA<sub>2</sub>-AA<sub>3</sub>-AA<sub>4</sub>-AA<sub>5</sub>-AA<sub>6</sub>-AA<sub>7</sub>-AA<sub>8</sub>-AA<sub>9</sub>-AA<sub>10</sub>-AA<sub>11</sub>-AA<sub>12</sub>-AA<sub>13</sub> (SEQ ID NO:464).

88. (Amended) The telomerase reverse transcriptase polypeptide of Claim 87, wherein: AA<sub>1</sub> is a hydrophobic amino acid or cysteine; AA<sub>2</sub> is any amino acid; AA<sub>3</sub> is threonine, lysine, tyrosine or glutamic acid; AA<sub>4</sub> is a hydrophobic amino acid or aspartic acid; AA<sub>5</sub> is aspartic acid; AA<sub>6</sub> is a hydrophobic amino acid or tyrosine; AA<sub>7</sub> is glutamic acid, lysine or glycine; AA<sub>8</sub> is any amino acid; AA<sub>9</sub> is cysteine or alanine; AA<sub>10</sub> is a hydrophobic amino acid, tyrosine or phenylalanine; AA<sub>11</sub> is aspartic acid or phenylalanine; AA<sub>12</sub> is serine or threonine; and, AA<sub>13</sub> is a hydrophobic amino acid (SEQ ID NO:469).

89. (Amended) The telomerase reverse transcriptase polypeptide of claim 88, wherein: AA<sub>1</sub> is phenylalanine; AA<sub>2</sub> is any amino acid; AA<sub>3</sub> is threonine or lysine; AA<sub>4</sub> is methionine, phenylalanine, valine; AA<sub>5</sub> is aspartic acid; AA<sub>6</sub> is isoleucine or valine; AA<sub>7</sub> is glutamic acid or lysine; AA<sub>8</sub> is any amino acid; AA<sub>9</sub> is cysteine or alanine; AA<sub>10</sub> is tyrosine; AA<sub>11</sub> is aspartic acid; AA<sub>12</sub> is serine or threonine; and, AA<sub>13</sub> is isoleucine or valine (SEQ ID NO:470).

90. The telomerase reverse transcriptase polypeptide of claims 86 to 89 wherein the polypeptide is produced by recombinant means.

91. An isolated nucleic acid encoding the polypeptide of claims 86 to 89 or variants thereof.
92. (Amended) An isolated, substantially pure, or recombinant telomerase reverse transcriptase polypeptide, said polypeptide comprising an amino acid sequence with significant sequence identity to a motif comprising :  
Trp-R<sub>1</sub>-X<sub>7</sub>-R<sub>1</sub>-R<sub>1</sub>-R<sub>2</sub>-X-Phe-Phe-Tyr-X-Thr-Glu-X<sub>8,9</sub>-R<sub>3</sub>-R<sub>3</sub>-Arg-R<sub>4</sub>-X<sub>2</sub>-Trp (SEQ ID NOS:16 and 17)  
where X is any amino acid and a subscript refers to the number of consecutive residues, R<sub>1</sub> is leucine or isoleucine, R<sub>2</sub> is glutamine or arginine, R<sub>3</sub> is phenylalanine or tyrosine, and R<sub>4</sub> is lysine or histidine.
93. (Amended) An isolated, substantially pure or recombinant nucleic acid that encodes a telomerase reverse transcriptase polypeptide, said polypeptide comprising an amino acid sequence with significant sequence identity to a motif comprising:  
Trp-R<sub>1</sub>-X<sub>7</sub>-R<sub>1</sub>-R<sub>1</sub>-R<sub>2</sub>-X-Phe-Phe-Tyr-X-Thr-Glu-X<sub>8,9</sub>-R<sub>3</sub>-R<sub>3</sub>-Arg-R<sub>4</sub>-X<sub>2</sub>-Trp (SEQ ID NOS:16 and 17)  
where X is any amino acid and a subscript refers to the number of consecutive residues, R<sub>1</sub> is leucine or isoleucine, R<sub>2</sub> is glutamine or arginine, R<sub>3</sub> is phenylalanine or tyrosine, and R<sub>4</sub> is lysine or histidine.
94. An isolated telomerase reverse transcriptase polypeptide comprising an amino acid sequence comprising a motif of contiguous amino acids selected from the group consisting of: Motif T of Figure 55, Motif 1 of Figure 55, Motif 2 of Figure 55, Motif A of Figure 55, Motif B' of Figure 55, Motif C of Figure 55, Motif D of Figure 55 and Motif E of Figure 55.
95. (Amended) An isolated telomerase reverse transcriptase polypeptide comprising an amino acid sequence with significant sequence identity to a motif selected from the group consisting of:  
Motif T, comprising the sequence W-X<sub>12</sub>-FFY-X-T-E-X<sub>10-11</sub>-R-X<sub>3</sub>-W-X<sub>7</sub>-I (SEQ ID NOS:119 and 120), or,  
W-L-X-Y-X-X-h-h-X-h-h-X-p-F-F-Y-X-  
T-E-X-p-X-X-X-p-X-X-X-Y-X-R-K-X-X-W (SEQ ID NO:473);  
Motif T' comprising the sequence E-X<sub>2</sub>-V-X (SEQ ID NO:121);  
Motif 1, comprising the sequence h-R-h-X-P-K (SEQ ID NO:478), or X<sub>3</sub>-R-X<sub>2</sub>-P-K-X<sub>3</sub> (SEQ ID NO:479);  
Motif 2, comprising the sequence X-R-X-I-X (SEQ ID NO:143) or (F/L)-R-h-I-X<sub>2</sub>-h (SEQ ID NO:479);  
Motif A, comprising the sequence X<sub>4</sub>-F-X<sub>3</sub>-D-X<sub>4</sub>-Y-D-X<sub>2</sub> (SEQ ID NO:144) , or, P-X-L-Y-F-h-X-h-D-h-X<sub>2</sub>- C-Y-D-X-I (SEQ ID NO:480);

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Motif B', comprising the sequence Y-X<sub>4</sub>-G-X<sub>2</sub>-Q-G-X<sub>3</sub>-S-X<sub>8</sub> (SEQ ID NO:146) , or, K-X-Y-X-Q-X<sub>2</sub>-G-I-P-Q-G-S-X-L-S-X-h-L (SEQ ID NO:474); and,  
Motif C, comprising the sequence X<sub>6</sub>-D-D-X-L-X<sub>3</sub> (SEQ ID NO:147), or  
L-L-R-L-X-D-D-X-L-h-I-T (SEQ ID NO:481);  
wherein X is any amino acid, X<sub>2</sub> is any two amino acids, X<sub>3</sub> is any three amino acids, and the like.

96. (Amended) An isolated telomerase reverse transcriptase polypeptide comprising an amino acid sequence with significant sequence identity to a motif comprising W(L/I)XXXXhXhXh(Q/R)XFFYXTEXXXXXXXXXX(F/Y)(F/Y)RXXXWXX(L/I)X XHXIXXXX(K/M) (SEQ ID NO:471), wherein X is any amino acid and wherein h is a hydrophobic amino acid.

97. (Amended) An isolated telomerase reverse transcriptase polypeptide comprising an amino acid sequence with significant sequence identity to a motif comprising the sequence FFYXTE (SEQ ID NO:71), wherein X is any amino acid.

98. (Amended) An isolated telomerase reverse transcriptase polypeptide comprising an amino acid sequence with significant sequence identity to a motif comprising the sequence hRhIPK (SEQ ID NO:367), wherein h is a hydrophobic amino acid.

99. (Amended) An isolated telomerase reverse transcriptase polypeptide comprising an amino acid sequence with significant sequence identity to a motif comprising the sequence hXXXXhRhIPK (SEQ ID NO:472), wherein h is a hydrophobic amino acid and wherein X is any amino acid.

100. (Amended) An isolated telomerase reverse transcriptase polypeptide comprising an amino acid sequence with significant sequence identity to a motif comprising the sequence KXYXQXXGIPQGSXLSXhLXXhXYXDL (SEQ ID NO:474), wherein h is a hydrophobic amino acid and wherein X is any amino acid.

101. (Amended) An isolated telomerase reverse transcriptase polypeptide comprising an amino acid sequence with significant sequence identity to a motif comprising the sequence (L/I)(L/M)(R/K)(L/V)XDD(F/Y)Lh(I/V)(T/S) (SEQ ID NO:475) wherein h is a hydrophobic amino acid and wherein X is any amino acid.

102. An isolated telomerase reverse transcriptase polypeptide comprising comprising an amino acid sequence with significant sequence identity to a motif comprising the sequence (NH<sub>2</sub>)- X<sub>300-600</sub>-W-X<sub>12</sub>-FFY-X-TE-X<sub>10-11</sub>-R-X<sub>3</sub>-W-X<sub>7</sub>-I-X<sub>5-20</sub>-E-X<sub>2</sub>-V-X-X<sub>5-20</sub>-X<sub>3</sub>-R-X<sub>2</sub>-PK-X<sub>4</sub>-<sub>10</sub>-R-X-I-X-X<sub>60-80</sub>-X<sub>4</sub>-F-X<sub>3</sub>-D-X<sub>4</sub>-YD-X<sub>2</sub>-X<sub>80-130</sub>-Y-X<sub>4</sub>-G-X<sub>2</sub>-QG-X<sub>3</sub>-S-X<sub>8</sub>-X<sub>5-35</sub>-X<sub>6</sub>-DD-X-L-X<sub>3</sub>-X<sub>10-20</sub>-X<sub>12</sub>-K, wherein h is a hydrophobic amino acid and wherein X is any amino acid.

Exhibit A  
U.S. Patent Application Serial No. 08/974,584  
For: TELOMERASE REVERSE TRANSCRIPTASE  
Filed: November 19, 1997  
Inventors: Cech, et al.

103. The polypeptide of claims 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, or 102, comprising an amino acid sequence of a naturally occurring telomerase reverse transcriptase protein.

104. The telomerase reverse transcriptase protein of claim 103 selected from the group of organisms consisting of Euplotes, Tetrahymena, fungi, Schizosaccharomyces, yeast, Oxytricha, mouse and mammals.

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